

IN THE CLAIMS

1. (original) A method for making an infectious adenovirus which comprises contacting a cell or introducing into a cell:

(a) either (i) a first nucleic acid sequence encoding adenovirus sequences which, in the absence of intermolecular recombination, are insufficient to encode an infectious, replicable or packageable adenovirus, said first nucleic acid sequence comprising at least one site-specific recombinase recognition target site which is recognized by a site-specific recombinase or (ii) a first nucleic acid sequence encoding adenovirus sequences which are sufficient to encode an infectious, replicable or packageable adenovirus and comprising at least one site-specific recombinase recognition target site which is recognized by a site-specific recombinase, wherein contact of said first nucleic acid with said site-specific recombinase results in excision of sequences from said first nucleic acid sequence such that, in the absence of intermolecular recombination, said adenovirus of (ii) is rendered replication or packaging defective;

(b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of adenoviral replication factors provided in trans or intermolecular recombination with said first nucleic acid sequence, are insufficient to encode an infectious, replicable or packageable adenovirus, said second nucleic acid sequence comprising at least one recombinase recognition target site sufficiently identical with said recombinase recognition target site in said first nucleic acid as to be recognized by the same site-specific recombinase which recognizes said site-specific recombinase recognition target site in said first nucleic acid;

whereby said first and said second nucleic acid sequences, in combination and following site-specific intermolecular recombination, result in production of an infectious adenovirus, and wherein a site-specific recombinase which recognizes said site-specific recombinase recognition target sites is either (i) expressed by a cell into which said first and said second nucleic acids are introduced, (ii) operatively encoded by said first nucleic acid, said second nucleic acid or both, or (iii) is provided in trans through expression from a third nucleic acid or is provided in trans as an active protein.

2. (original) The method according to claim 1 wherein said second nucleic acid sequence is a

2 plasmid comprising:

- 3 (i) all or most of the left ITR and the packaging signal contained within the leftmost
4 approximately 350 nt of the adenovirus genome;
5 (ii) a polycloning site or a foreign DNA or an expression cassette; and
6 (iii) a *lox P* site 3' of said polycloning site, foreign DNA, or an expression cassette.

1 3. (original) The method according to claim 1 wherein said first nucleic acid sequence is a
2 plasmid containing a circularized adenovirus DNA molecule encoding adenovirus
3 sequences which, in the absence of intermolecular recombination, are insufficient to
4 encode an infectious, replicable or packageable adenovirus.

1 4. (original) The method according to claim 3 wherein said plasmid includes a bacterial
2 origin of DNA replication and an antibiotic resistance gene for selection in bacteria.

1 5. (original) The method according to claim 3 wherein said adenovirus DNA has a deletion
2 of an adenoviral packaging signal, or wherein said packaging signal is flanked on either
3 side by at least one of said site-specific recombinase recognition sites.

1 6. (original) The method according to claim 5 wherein said adenovirus DNA comprises (i) a
2 deletion of, (ii) a modification in, or (iii) a flanking with a site-specific recombinase
3 recognition site of, an adenoviral gene selected from the group consisting of adenoviral
4 E1 sequences extending beyond said packaging signal, adenoviral fibre gene sequences,
5 adenoviral E3 gene sequences, adenoviral E4 gene sequences, and combinations thereof.

1 7. (original) A recombinant adenovirus vector system comprising:

- 2 (a) either (i) a first nucleic acid sequence encoding adenovirus sequences which, in the
3 absence of intermolecular recombination, are insufficient to encode an infectious,
4 replicable or packageable adenovirus, said first nucleic acid sequence comprising at least
5 one site-specific recombinase recognition target site which is recognized by a site-
6 specific recombinase or (ii) a first nucleic acid sequence encoding adenovirus sequences

7 which are sufficient to encode an infectious, replicable or packageable adenovirus and
8 comprising at least one site-specific recombinase recognition target site which is
9 recognized by a site-specific recombinase, wherein contact of said first nucleic acid with
10 said site-specific recombinase results in excision of sequences from said first nucleic acid
11 sequence such that, in the absence of intermolecular recombination, said adenovirus of
12 (ii) is rendered replication or packaging defective;

- 13 (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence of
14 adenoviral replication factors provided in trans or intermolecular recombination with said
15 first nucleic acid sequence, are insufficient to encode an infectious, replicable or
16 packageable adenovirus, said second nucleic acid sequence comprising at least one
17 recombinase recognition target site sufficiently identical with said recombinase
18 recognition target site in said first nucleic acid as to be recognized by the same site-
19 specific recombinase which recognizes said site-specific recombinase recognition target
20 site in said first nucleic acid;

21 whereby said first and said second nucleic acid sequences, in combination and following site-
22 specific intermolecular recombination, result in production of an infectious adenovirus, and
23 wherein a site-specific recombinase which recognizes said site-specific recombinase recognition
24 target sites is either (i) expressed by a cell into which said first and said second nucleic acids are
25 introduced, (ii) operatively encoded by said first nucleic acid, said second nucleic acid or both, or
26 (iii) is provided in trans through expression from a third nucleic acid or is provided in trans as an
27 active protein.

1 8. (original) The recombinant adenovirus vector system of claim 7 wherein said cell further
2 expresses adenoviral E1.

1 9. (currently amended) The recombinant adenovirus vector system of claim 7 wherein said
2 first [plasmid] nucleic acid sequence and said second [plasmid] nucleic acid sequence are
3 cotransfected into said cell to produce an infectious virus vector comprising a left end, a
4 polycloning site, foreign DNA, or an expression cassette derived from said second
5 [plasmid] nucleic acid sequence, joined to the remaining portion of the viral DNA

6 derived from said first [plasmid] nucleic acid sequence.

1 10. (original) The recombinant adenovirus vector system according to claim 7 wherein said
2 first nucleic acid sequence comprises a recombinase recognition site and a deletion in the
3 adenoviral fibre gene.

1 11. (currently amended) A kit for construction of a recombinant adenovirus vector[s]
2 comprising:
3 (a) either (i) a first nucleic acid sequence encoding adenovirus sequences which, in the
4 absence of intermolecular recombination, are insufficient to encode an infectious,
5 replicable or packageable adenovirus, said first nucleic acid sequence comprising at least
6 one site-specific recombinase recognition target site which is recognized by a site-
7 specific recombinase or (ii) a first nucleic acid sequence encoding adenovirus sequences
8 which are sufficient to encode an infectious, replicable or packageable adenovirus and
9 comprising at least one site-specific recombinase recognition target site which is
10 recognized by a site-specific recombinase, wherein contact of said first nucleic acid with
11 said site-specific recombinase results in excision of sequences from said first nucleic acid
12 sequence such that, in the absence of intermolecular recombination, said adenovirus of
13 (ii) is rendered replication or packaging defective;
14 (b) a second nucleic acid sequence encoding adenovirus sequences which, in the absence
15 of adenoviral replication factors provided in trans or intermolecular recombination with
16 said first nucleic acid sequence, are insufficient to encode an infectious, replicable or
17 packageable adenovirus, said second nucleic acid sequence comprising at least one
18 recombinase recognition target site sufficiently identical with said recombinase
19 recognition target site in said first nucleic acid as to be recognized by the same site-
20 specific recombinase which recognizes said site-specific recombinase recognition target
21 site in said first nucleic acid; and
22 (c) a cell wherein, when said first nucleic acid sequence and said second nucleic acid
23 sequence are cotransfected and recombined through the action of a recombinase which
24 recognizes said recombinase recognition sites, [to produce a] said recombinant adenovirus

25 vector, packaged and infectious, is constructed [adenovirus vector].

1 12. (currently amended) The kit according to claim 11 wherein said cell of (c) is selected
2 from the group consisting of 293 cells expressing Cre, PER-C6 cells expressing Cre, and
3 911 cells expressing Cre, and wherein said recombinase recognition sites are lox sites.

1 13. (currently amended) A[The] recombinant adenovirus vector system [according to claim
2 7] comprising:

3 (a) either (i) a first nucleic acid sequence encoding adenovirus sequences which, in
4 the absence of intermolecular recombination, are insufficient to encode an
5 infectious, replicable or packageable adenovirus, said first nucleic acid sequence
6 comprising at least one site-specific recombinase recognition target site which is
7 recognized by a site-specific recombinase or (ii) a first nucleic acid sequence
8 encoding adenovirus sequences which are sufficient to encode an infectious,
9 replicable or packageable adenovirus, said first nucleic acid sequence comprising
10 (A) at least one restriction enzyme recognition site such that upon restriction of
11 said nucleic acid with a restriction enzyme which recognizes said site, a site-
12 specific recombinase recognition target site remains intact, but said adenovirus of
13 (ii) is rendered replication or packaging deficient, or (B) wherein said nucleic acid
14 comprises at least one site-specific recombinase recognition site which is
15 recognized by a site-specific recombinase, wherein contact of said first nucleic
16 acid with said site-specific recombinase results in excision of sequences from said
17 first nucleic acid sequence such that, in the absence of intermolecular
18 recombination, said adenovirus of (ii) is rendered replication or packaging
19 defective;

20 (b) a second nucleic acid sequence encoding adenovirus sequences which, in the
21 absence of adenoviral replication factors provided in trans or intermolecular
22 recombination with said first nucleic acid sequence, are insufficient to encode an
23 infectious, replicable or packageable adenovirus, said second nucleic acid
24 sequence comprising at least one recombinase recognition target site sufficiently

25 identical with said recombinase recognition target site in said first nucleic acid as
26 to be recognized by the same site-specific recombinase which recognizes said
27 site-specific recombinase recognition target site in said first nucleic acid;
28 wherein said first and said second nucleic acid sequences, in combination and following
29 site-specific intermolecular recombination, result in production of an infectious
30 adenovirus, and wherein a site-specific recombinase which recognizes said site-specific
31 recombinase recognition target sites is either (i) expressed by a cell into which said first
32 and said second nucleic acids are introduced, (ii) operatively encoded by said first nucleic
33 acid, said second nucleic acid or both, or (iii) is provided in trans through expression
34 from a third nucleic acid or is provided in trans as an active protein.

14. (new) The method according to claim 1 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of a loxP sequence, and wherein said site-specific recombinase is comprised of Cre.
15. (new) The method according to claim 1 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of an frt sequence, and wherein said site-specific recombinase is comprised of FLP.
16. (new) The recombinant adenovirus vector system according to claim 7 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of a loxP sequence, and wherein said site-specific recombinase is comprised of Cre.
17. (new) The recombinant adenovirus vector system according to claim 7 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and

said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of an *frt* sequence, and wherein said site-specific recombinase is comprised of FLP.

18. (new) The kit according to claim 11 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of a *loxP* sequence, and wherein said site-specific recombinase is comprised of Cre.
19. (new) The kit according to claim 11 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of an *frt* sequence, and wherein said site-specific recombinase is comprised of FLP.
20. (new) The recombinant adenovirus vector system according to claim 7 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of a *loxP* sequence, and wherein said site-specific recombinase is comprised of Cre.
21. (new) The recombinant adenovirus vector system according to claim 7 wherein said at least one site-specific recombinase recognition target site of said first nucleic acid sequence and said at least one site-specific recombinase recognition target site of said first nucleic acid sequence each is comprised of an *frt* sequence, and wherein said site-specific recombinase is comprised of FLP.